A ^{31}P -n.m.r. study of the acute effects of β -blockade on the bioenergetics of skeletal muscle during contraction

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1. The effects of β -adrenoceptor antagonist administration on skeletal muscle contractile performance and bioenergetics *in vivo* have been investigated during unilateral sciatic nerve stimulation in the rat. 2. Two muscle stimulation protocols have been used: supramaximal stimulation at 4 Hz, or incremental supramaximal stimulation at 1, 2 and 4 Hz. Changes in high-energy phosphate concentrations were followed using ^{31}P -n.m.r., and gastrocnemius muscle twitch characteristics were monitored continuously. 3. Under all conditions investigated, DL-propranolol administration (2.5 mg/kg body wt.) caused a significant decrease in cyclic AMP concentrations in resting and stimulated gastrocnemius muscle, prevented an increase in heart rate upon muscle stimulation, but did not affect plasma glucose, fatty acid or lactate concentrations in comparison with values obtained in control experiments. 4. Administration of DL-propranolol 5 min or 35 min before unilateral stimulation of 4 Hz had no effect on changes in muscle phosphocreatine, ATP or P_i concentrations, intracellular pH or contractile performance. 5. In contrast, animals receiving DL-propranolol 5 min before unilateral stimulation of 1, 2 and 4 Hz showed a significant deterioration in gastrocnemius muscle tension development during 2 and 4 Hz stimulation compared with control animals. Concurrent with this change in contractile performance was a higher muscle concentration of phosphocreatine, a lower concentration of P_i and no significant change in intramuscular pH compared with control experiments. 6. The changes in muscle performance and bioenergetics observed during the incremental stimulation protocol were not observed when D-propranolol was administered and could be completely circumvented by a short period of muscle stimulation of 4 Hz prior to initiation of the incremental stimulation protocol. 7. Mechanisms are discussed which may account for the failure of gastrocnemius muscle to generate the expected force during the incremental stimulation protocol in the presence of β -blockade.

INTRODUCTION

Upon initiation of muscle contraction, the rates of glycolysis and pyruvate oxidation increase to accommodate the increased demand for high-energy phosphate. Glycogen stored in the muscle is considered to be a major source of fuel (Armstrong *et al.*, 1974). Two stimuli of muscle glycogenolysis are known. During muscle contraction calcium is released from the sarcoplasmic reticulum, causing a co-ordinated activation of cytosolic and mitochondrial energy-producing pathways including activation of phosphorylase *b* kinase, pyruvate dehydrogenase phosphatase and 2-oxoglutarate dehydrogenase (Soderling & Park, 1974; Cohen, 1982; Denton & McCormack, 1985). In addition, plasma catecholamines may increase during exercise (Galbo *et al.*, 1975; Christensen *et al.*, 1979); causing, via β -adrenoceptor activation, an increase in cellular cyclic AMP concentration and a net conversion of phosphorylase *b* to phosphorylase *a* (Soderling & Park, 1974; Cohen, 1982). The precise interaction between Ca^{2+} and cyclic AMP-mediated cellular responses is not clearly understood. Although the importance of changes in cytosolic and mitochondrial concentrations of Ca^{2+} during muscle contraction are now appreciated at a molecular level (Fischer *et al.*, 1971; Cohen, 1982; Denton & McCormack, 1985), the role of changes of plasma catecholamines in regulating substrate delivery and

intramuscular control of metabolic fluxes has proved to be a controversial and contentious area of exercise biochemistry which has been exacerbated by the likelihood that the relative importance of hormonal and Ca^{2+} stimuli may vary depending on the duration and intensity of exercise (Richter *et al.*, 1981; Gorski & Pietrzyk, 1982).

Elegant studies in which the effects of adrenomedullation in combination with chemical or surgical sympathectomy have been investigated *in vivo* have suggested that catecholamines, in particular adrenaline derived from the adrenal medulla, play a major role in the regulation of muscle glycogen breakdown during exercise (Galbo *et al.*, 1978; Richter *et al.*, 1981). Further work using the isolated perfused hindquarter preparation has suggested that the effect of adrenaline is exerted directly on the contracting muscle mass (Richter *et al.*, 1982a,b).

An alternative strategy, widely applied to animal and human studies, is the use of β -adrenoceptor antagonists to abolish β -adrenoceptor stimulation during exercise. Generally, administration of β -adrenoceptor antagonists, either acutely or chronically, impairs muscle performance (Issekutz, 1978; Lundborg *et al.*, 1981; Twentymen *et al.*, 1981) although notable exceptions have been reported (Nazar *et al.*, 1972).

In the present study, we have used ^{31}P -n.m.r. to follow muscle high-energy phosphate utilization and intracellular changes in pH during acute β -blockade *in vivo*.

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METHODS

Animal preparation

Male Wistar rats (200–220 g) were obtained from OLAC (1976) Ltd., Bicester, Oxon, U.K., and were maintained in the Department's animal facility with free access to food and water for at least 5 days before use.

Anaesthesia was induced with pentobarbital (60 mg/kg, intraperitoneal). A cannula containing heparinized saline was introduced into the tail artery (for monitoring blood-pressure and administration of propranolol). The left sciatic nerve was exposed, two Teflon-coated silver electrodes were sewn into place in contact with the nerve and the wound was sutured. Brass pins were forced through the knee and ankle joints and the limb was placed in a specially designed perspex cradle. The distal tendon of the gastrocnemius muscle was attached to a force transducer and the muscle was adjusted to the optimal length for maximal isometric contraction (Shoubridge *et al.*, 1984; Challiss *et al.*, 1986). The whole apparatus was placed in the vertical bore of the magnet. Rectal temperature was monitored and maintained at $37 \pm 0.5^\circ\text{C}$ by blowing warm air over the animal. Anaesthesia was maintained using 0.5–1% halothane in $\text{N}_2\text{O}/\text{O}_2$ (1:1) delivered via a face mask.

N.m.r. spectroscopy

All experiments were performed at 73.84 MHz in a vertical wide bore 4.3 T magnet. A three-turn surface coil (14 mm outer diameter) was placed over the gastrocnemius muscle and served as both transmitter and receiver. The surface coil was tuned to the ^{31}P resonance frequency.

Magnetic field homogeneity was adjusted while observing the proton signal of muscle water. Spectra were collected as 1024 data points using a sweep-width of ± 1500 Hz, a pulse-width of $15\ \mu\text{s}$ (nominal tip-angle of 70° at centre of coil) and a recycle time of 2 s (10 s for fully-relaxed spectra). Spectra were the result of 64 transients and were zero-filled. The pH was calculated from the chemical shift of P_i relative to phosphocreatine (Gadian *et al.*, 1979).

At the beginning of each experiment, a fully-relaxed spectrum was collected for quantification of phosphorus-containing metabolites. At least three further resting spectra were collected (2 s recycle time) and saturation factors calculated (Taylor *et al.*, 1983). Muscle contraction was caused by sciatic nerve stimulation (50 μs duration, 45 V). Muscle contraction was optimized during the shimming procedure by applying two or three single pulses. Experiments where $> 50\text{V}$ (50 μs duration) did not give maximum contraction at any time during the protocol were discarded ($< 5\%$ of all experiments performed).

Physiological studies

Identical experiments to those done in the magnet were carried out in a rigid animal frame to optimize acquisition of physiological information on muscle performance and to allow efficient freeze-clamping of gastrocnemius muscle samples for biochemical analysis. In addition, in some experiments a cannula was placed in the right iliac artery just below the aortic bifurcation to allow continuous monitoring of blood pressure and heart rate or for sampling of arterial blood for metabolite analysis.

Muscle stimulation protocols

Two protocols were used: sciatic nerve stimulation at a frequency of 4 Hz for 32 min (15×64 transients, 2 s recycle time) or continuous sciatic nerve stimulation at 1, 2 and 4 Hz, each for approx. 16 min (7×64 transients, 2 s recycle time). In magnet experiments, after completion of either protocol a recovery period of approx. 15 min was allowed before collection of the final fully-relaxed spectrum. The animal was removed from the probe and the stimulated and contralateral gastrocnemius muscles exposed and freeze-clamped for subsequent analysis of ATP and total creatine (creatine-plus-phosphocreatine) concentrations.

Metabolite analysis

Adenine nucleotides, IMP and creatine metabolites were determined by using the h.p.l.c. method of Harmsen *et al.* (1982). Glucose, lactate and fatty acids were determined by the methods of Bergmeyer *et al.* (1974), Engel & Jones (1978) and Okabe (1980), respectively. Glycogen was enzymically degraded to glucose using amyloglucosidase and the glucose concentration determined (Keppler & Decker, 1974). Cyclic AMP was measured by using the radioimmunoassay package supplied by Amersham International.

Materials

All biochemicals were obtained from Boehringer or Sigma. D-Propranolol was kindly given by I.C.I. Pharmaceutical Division, Macclesfield, Cheshire, U.K.

RESULTS

Quantification of ^{31}P -n.m.r. spectra of resting muscle

Typical ^{31}P -n.m.r. spectra of the rat gastrocnemius muscle at rest are shown in Figs. 1 and 2. From the dimensions of the radiofrequency coil used it can be estimated that signal was acquired from a volume of approx. 0.7 cm^3 of skeletal muscle; this was predominantly the medial head of the gastrocnemius with a small contribution from the plantaris muscle (Shoubridge *et al.*, 1984).

There is good evidence that chemically determined ATP is entirely n.m.r.-visible (Dawson *et al.*, 1980; Meyer *et al.*, 1982); thus, using the respective chemically determined values for ATP and total creatine for each animal studied and assuming that the volume of intracellular water is 0.67 ml/g wet wt. in the muscle volume interrogated (Sahlin, 1978; Sjogaard & Saltin, 1982), the concentrations of phosphocreatine and P_i can be calculated from the fully-relaxed ^{31}P -n.m.r. spectra.

The lower spectra ('before' in Figs. 1 and 2) were obtained from a control animal (a) or from an animal receiving 2.5 mg of DL-propranolol/kg body wt. 5 min prior to spectral acquisition (b). Propranolol at doses of 0.5–5 mg/kg had no effect on resting phosphocreatine, ATP or P_i concentrations or on intramuscular pH. At a dose of 2.5 mg/kg, DL-propranolol had no effect on resting heart-rate or systolic blood pressure (measured by catheterization of the right iliac artery; Table 1). Upon stimulation, heart rate and systolic blood pressure increased in control animals; however, no increase in heart rate was observed in animals receiving the β -blocker and the rise in systolic blood pressure was decreased such

Fig. 1. ^{31}P -n.m.r. spectra taken before, during and after stimulation of rat gastrocnemius muscle at 4 Hz

Isometric contraction of gastrocnemius muscle was induced by sciatic nerve stimulation with supramaximal (45 V, 50 μs duration) pulses at 4 Hz for 32 min. Spectra shown during stimulation were acquired at 2–4 min, 14–16 min and 26–28 min for a control animal (a) or an animal receiving DL-propranolol 5 min prior to commencing stimulation (b).

Fig. 2. ^{31}P -n.m.r. spectra taken before, during and after stimulation of rat gastrocnemius muscle at 1, 2 and 4 Hz

Isometric contraction of gastrocnemius muscle was induced by sciatic nerve stimulation with supramaximal (45 V, 50 μs duration) pulses at 1, 2 and 4 Hz for 15 min periods. Spectra shown during stimulation were acquired at 2–4 min and 11–13 min at each stimulation frequency for a control animal (a) or an animal receiving DL-propranolol 5 min prior to commencing stimulation (b).

Table 1. Changes in heart rate, systolic blood pressure and plasma concentrations of glucose, lactate and fatty acids during unilateral sciatic nerve stimulation at 1, 2 and 4 Hz and during recovery

Catheterization and sciatic nerve stimulation were carried out as described in the Methods section. Heart rate and systolic blood pressure were monitored continuously. Blood samples (200 μ l) were taken at rest (before and 4 min after intra-arterial infusion of DL-propranolol) and after 10 min stimulation at each frequency; a final blood sample was taken 15 min after termination of stimulation. Plasma glucose, lactate and fatty acids were determined as described in the Methods section. Results are presented as means $\pm$ S.E.M. for at least four separate experiments. Statistical significance was evaluated (Student's *t*-test for unpaired observations) between animals receiving either 2.5 mg of propranolol/kg body wt. or saline vehicle only and is indicated as: **P* < 0.05, ***P* < 0.01.

	Heart rate (beats/min)	Blood pressure (systolic; mmHg)	Glucose (mM)	Lactate (mM)	Fatty acid (mM)
Rest					
Control	393 $\pm$ 15	122 $\pm$ 10	7.6 $\pm$ 0.7	1.4 $\pm$ 0.3	0.26 $\pm$ 0.04
Pre- β -blocker	395 $\pm$ 15	123 $\pm$ 13	7.3 $\pm$ 0.7	1.3 $\pm$ 0.2	0.24 $\pm$ 0.03
Post- β -blocker	390 $\pm$ 13	121 $\pm$ 9	7.7 $\pm$ 0.3	1.3 $\pm$ 0.1	0.24 $\pm$ 0.02
Stimulation					
1 Hz					
Control	423 $\pm$ 10	141 $\pm$ 7	8.4 $\pm$ 0.3	1.7 $\pm$ 0.1	—
β -Blocker	388 $\pm$ 13*	136 $\pm$ 6	8.0 $\pm$ 0.2	1.5 $\pm$ 0.1	—
2 Hz					
Control	430 $\pm$ 10	151 $\pm$ 6	8.0 $\pm$ 0.8	1.7 $\pm$ 0.2	—
β -Blocker	385 $\pm$ 11**	142 $\pm$ 5	8.8 $\pm$ 0.3	1.7 $\pm$ 0.3	—
4 Hz					
Control	450 $\pm$ 22	158 $\pm$ 7	8.9 $\pm$ 0.3	1.8 $\pm$ 0.3	0.26 $\pm$ 0.06
β -Blocker	384 $\pm$ 14*	139 $\pm$ 5*	8.9 $\pm$ 0.4	2.0 $\pm$ 0.4	0.33 $\pm$ 0.10
Recovery					
Control	400 $\pm$ 20	130 $\pm$ 4	7.9 $\pm$ 0.9	1.1 $\pm$ 0.2	0.25 $\pm$ 0.06
β -Blocker	358 $\pm$ 15	128 $\pm$ 8	7.8 $\pm$ 0.6	1.0 $\pm$ 0.2	0.28 $\pm$ 0.07

that at a stimulation frequency of 4 Hz significant differences were observed for control and β -blocked animals (Table 1). In addition, the initial twitch-tension was unaffected by propranolol treatment [initial control twitch-tension 2.08 ± 0.28 (12) g/g body wt. versus 2.02 ± 0.17 (12) g/g body wt. for propranolol-treated animals].

Metabolic and physiological response to muscle stimulation at 4 Hz

A series of spectra acquired during sciatic nerve stimulation at 4 Hz for 32 min are shown in Fig. 1. The quantitative data obtained from such spectra are shown in Fig. 3, which also illustrates the physiological performance of the gastrocnemius muscle during stimulation. From Fig. 3 it can be seen that upon stimulation at 4 Hz the phosphocreatine concentration decreased by about 80% and the intracellular pH fell to 6.45 within the first 5 min of stimulation. After 15 min of stimulation, phosphocreatine concentration was constant at 30–40% of the initial resting concentration, the intramuscular pH had risen to 6.95 and the work performed by the gastrocnemius muscle had achieved a constant level at about 65% of the initial twitch-tension.

Administration of 2.5 mg of DL-propranolol/kg body wt. 5 min prior to muscle stimulation had no effect on changes in phosphocreatine concentration, pH or muscle performance during stimulation at 4 Hz (Fig. 3). In a similar series of experiments, to allow direct temporal comparison with the incremental protocol (see below), it was shown that administration of propranolol 35 min prior to muscle stimulation also had no effect on the n.m.r.-determined parameters or on muscle perfor-

mance compared with experiments performed on control animals (results not shown).

In a parallel series of experiments to complement ^{31}P -n.m.r. data, gastrocnemius muscle of animals receiving 2.5 mg of DL-propranolol/kg or saline vehicle were stimulated at 4 Hz for 5 min, at which time the stimulated and contralateral gastrocnemius muscles were freeze-clamped. The effects of stimulation on muscle metabolite concentrations are reported in Table 2. Muscle contraction at 4 Hz for 5 min in control animals caused a 64% decrease in phosphocreatine concentration and a 26% decrease in ATP with concomitant increases in AMP and IMP concentrations; muscle lactate concentration was elevated 4-fold and muscle glycogen was depleted by about 50%. Similar changes occurred in the stimulated gastrocnemius muscle of animals receiving propranolol prior to stimulation (Table 2). The levels of cyclic AMP measured in muscles of animals undergoing 5 min of unilateral muscle stimulation were increased by 70% compared with levels measured in gastrocnemius muscle taken from anaesthetized animals not prepared for muscle stimulation [221 ± 34 (8) pmol/g of tissue]. Therefore, the only significant difference between measured gastrocnemius muscle metabolites of the two experimental groups was that cyclic AMP concentrations were statistically significantly decreased in both the stimulated and contralateral muscles of animals receiving the β -blocker (Table 2). The values measured in stimulated muscle of animals receiving propranolol were similar to those found in muscle of non-stimulated control animals.

Therefore, in summary, acute β -blockade lowered the gastrocnemius muscle concentration of cyclic AMP in

Fig. 3. Changes in twitch-tension, intracellular pH and phosphocreatine concentration in gastrocnemius muscle during stimulation at 4 Hz and during recovery in control animals (●) and those receiving DL-propranolol (■)

Values are means $\pm$ S.E.M. for eight control animals and nine animals receiving β -blocker.

both the contracting and contralateral muscle, but this had no effect on measured physiological or biochemical parameters.

Metabolic and physiological response to incremental muscle stimulation

A series of spectra acquired during sciatic nerve stimulation at 1, 2 and 4 Hz (about 15 min at each frequency) are shown in Fig. 2. The quantitative data obtained from such spectra are shown in Fig. 4, which also illustrates the physiological performance of gastrocnemius muscle during stimulation. For control animals, stimulation at 1 Hz caused an initial potentiation of twitch-tension followed by a decline in muscle performance such that at the end of the 4 Hz stimulation period the twitch-tension was $64 \pm 2\%$ (Fig. 4); this was similar to the steady state achieved in gastrocnemius muscle stimulated only at 4 Hz (Fig. 3). Muscle performance in animals receiving DL-propranolol is also shown in Fig. 4. The initial potentiation of twitch-tension was observed; however, twitch-tension declined more rapidly and statistically significant differences were evident upon stimulation at 2 and 4 Hz (Fig. 4), and at the end of the period of stimulation at 4 Hz twitch-tension was $46 \pm 4\%$ of the initial value.

The initial decrease in phosphocreatine concentration upon stimulation at 1 Hz was similar in animals receiving DL-propranolol or saline (Fig. 4); however, upon increasing the stimulation frequency to 2 Hz and subsequently to 4 Hz smaller changes in phosphocreatine concentration were seen in animals receiving β -blocker. At the end of the stimulation period the phosphocreatine concentration was 8.2 ± 0.9 (8) $\mu\text{mol/g}$ for gastrocnemius muscles of control animals and 13.6 ± 0.6 (9) $\mu\text{mol/g}$ for animals receiving DL-propranolol (Fig. 4). During incremental stimulation at 1, 2 and 4 Hz, no dramatic changes in intramuscular pH were observed for either experimental group (Fig. 4); after 4 min at a stimulation frequency of 4 Hz the respective pH values were 6.88 ± 0.03 (8) and 6.89 ± 0.02 (9) for experiments performed in the absence and presence of β -blockade.

Table 2. Effects of acute DL-propranolol administration on changes in gastrocnemius muscle metabolites during supramaximal isometric contraction at 4 Hz

DL-Propranolol was administered by intra-arterial infusion 5 min prior to commencing muscle stimulation. The gastrocnemius muscles were freeze-clamped after 5 min of unilateral muscle stimulation at 4 Hz. Results are presented as means $\pm$ S.E.M. for at least five separate experiments. Metabolite concentrations are expressed as $\mu\text{mol/g}$ of tissue, except ^a μmol of glucosyl equiv./h per g or ^b pmol/g of tissue. Statistical significance (Student's *t*-test for paired observations) is shown as: **P* < 0.05, ***P* < 0.01, ****P* < 0.001 for stimulated versus non-stimulated conditions. Statistical significance (Student's *t*-test for unpaired observations) is shown as: †*P* < 0.05 for propranolol-treated versus control values.

Metabolite	Control		†Propranolol	
	Non-stimulated	Stimulated	Non-stimulated	Stimulated
ATP	6.80 ± 0.42	$5.00 \pm 0.46^*$	7.08 ± 0.18	$5.42 \pm 0.47^{**}$
ADP	1.16 ± 0.06	1.32 ± 0.08	1.16 ± 0.03	1.34 ± 0.14
AMP	0.04 ± 0.004	$0.09 \pm 0.011^{**}$	0.04 ± 0.005	0.008 ± 0.019
IMP	0.07 ± 0.02	$0.75 \pm 0.11^{***}$	0.07 ± 0.02	$0.78 \pm 0.34^{***}$
Phosphocreatine	22.1 ± 1.5	$7.9 \pm 0.8^{**}$	24.4 ± 0.8	$9.1 \pm 1.2^{***}$
Creatine	7.5 ± 0.6	$15.2 \pm 1.1^{***}$	7.7 ± 1.2	$13.7 \pm 1.1^{***}$
Lactate	1.93 ± 0.13	$7.62 \pm 0.21^{***}$	1.95 ± 0.19	$6.88 \pm 0.81^{**}$
Glycogen ^a	35.3 ± 3.0	$16.5 \pm 1.9^{**}$	33.0 ± 2.0	$21.9 \pm 3.3^{**}$
Cyclic AMP ^b	421 ± 71	329 ± 29	$245 \pm 24^\dagger$	$233 \pm 31^\dagger$

Fig. 4. Changes in twitch-tension, intracellular pH and phosphocreatine concentration in gastrocnemius muscle during stimulation at 1, 2 and 4 Hz and during recovery in control animals (●) and those receiving DL-propranolol (■)

Values are means $\pm$ S.E.M. for eight control animals and nine animals receiving acute β -blockade. Statistical significance (Student's *t*-test for unpaired observations) is shown as: **P* < 0.05, ***P* < 0.01.

The comparable values of intracellular pH for muscles stimulated for 4 min at 4 Hz from rest were 6.42 ± 0.08 (7) and 6.43 ± 0.07 (9) respectively (Fig. 3).

Changes in plasma glucose, lactate and fatty acid concentrations during 1, 2 and 4 Hz stimulation are shown in Table 1. Sciatic nerve stimulation caused a modest increase in plasma glucose concentrations. No significant increases in plasma lactate or fatty acid concentrations were observed with incremental increases in stimulation frequency. Furthermore, the aerobic nature of this type of exercise was borne out by freeze-clamping studies and analysis of muscle metabolite concentrations in gastrocnemius muscle 5 min after increasing the stimulation frequency from 2 to 4 Hz (Table 3). For control animals, gastrocnemius muscle lactate concentration was increased by 65% in the exercising compared with the contralateral non-stimulated muscle.

Comparison of muscle metabolite concentrations in gastrocnemius muscles stimulated in the absence or

presence of DL-propranolol (Table 3) showed that ATP concentration was lower for both groups, being 33% lower than the pre-stimulation value for control animals and 24% lower for animals after β -blocker administration. Although AMP was raised in both groups, the concentration of IMP in stimulated muscle of control animals was 1.08 ± 0.19 (8) $\mu\text{mol/g}$ of tissue, but was significantly lower [0.42 ± 0.15 (8) $\mu\text{mol/g}$] in animals receiving DL-propranolol. In addition, the elevation in tissue lactate concentration and the reduction in glycogen concentration were less marked in animals receiving DL-propranolol (Table 3).

Clearly, the consequences of β -blockade vary with the type of exercise performed. When DL-propranolol is administered 5 or 35 min before unilateral isometric contraction of gastrocnemius muscle at 4 Hz, there is no detrimental effect on muscle performance, nor is there any apparent effect on cellular bioenergetics (Fig. 3 and Table 2). In contrast, DL-propranolol administration prior to initiating an incremental work protocol in which gastrocnemius is isometrically stimulated to contract at 1, 2 and 4 Hz does produce significant changes in muscle performance and cellular bioenergetics (Fig. 4 and Table 3).

With regard to the nature of the effect of DL-propranolol during the incremental exercise protocol, we have shown that D-propranolol given at 2.5 mg/kg body wt. had no effect on the performance of gastrocnemius muscle or the changes in phosphocreatine concentration (or pH) observed by ^{31}P -n.m.r. (i.e. all results in the presence of D-propranolol were similar to those obtained in its absence; results not shown). This strongly suggests that the effects observed are β -adrenoceptor-specific and are not due to other properties of propranolol (e.g. its lipophilicity).

A further series of experiments was carried out to attempt to establish the physiological and biochemical basis of the differences in the results obtained using the two stimulation protocols. It was found that in animals receiving β -blockade, exposure to a brief period of stimulation at 4 Hz (36 single isometric twitches in 9 s) followed by 10 min recovery and then initiation of the incremental 1, 2, 4 Hz protocol resulted in a loss of the β -blocker effect (Fig. 5 and Table 3); thus, in animals receiving DL-propranolol and exposed to a brief pre-stimulation, gastrocnemius muscle performance, changes in phosphocreatine concentration and pH were similar to results for control animals. The results of a freeze-clamping experiment in these pre-stimulated animals are shown in Table 3. For both experimental groups receiving acute β -blockade, cyclic AMP concentrations were decreased; in addition, the rise in IMP concentration in the stimulated muscle was depressed compared with the control results (Table 3). Comparing the data for the two groups receiving β -blockade, the main differences between metabolite concentrations were that the pre-stimulation group had significantly lower muscle glycogen and higher lactate concentrations (Table 3); however, using ^{31}P -n.m.r., no significant differences in intramuscular pH were observed during the incremental protocol for the pre-stimulated group (Fig. 5).

DISCUSSION

The use of β -adrenoceptor antagonists is a commonly employed pharmacological intervention in the treatment

Table 3. Effects of acute DL-propranolol administration on changes in gastrocnemius muscle metabolites during supramaximal isometric contraction at 4 Hz after previous incremental stimulation at 1 and 2 Hz

DL-Propranolol was administered by intra-arterial infusion. One group receiving propranolol was subjected to a short period (9 s) of muscle stimulation at 4 Hz followed by 10 min recovery before initiation of the 'step' protocol. The gastrocnemius muscles were stimulated at 1 and 2 Hz for 15 min at each frequency and freeze-clamped after 5 min of unilateral stimulation at 4 Hz. Results are presented as means $\pm$ S.E.M. for at least seven separate experiments. Metabolite concentrations are expressed as $\mu\text{mol/g}$ of tissue, except ^a μmol of glucosyl equiv./g of tissue or ^b pmol/g of tissue. Statistical significance (Student's *t*-test for paired observations) is shown as: **P* < 0.05, ***P* < 0.005 for stimulated versus non-stimulated conditions. Statistical significance (Student's *t*-test for unpaired observations) is shown as: †*P* < 0.05, ††*P* < 0.005 for propranolol-treated versus control values and as †††*P* < 0.01 for propranolol-treated versus pre-stimulation plus propranolol.

Metabolite	Control		+ Propranolol		Pre-stimulation + propranolol	
	Non-stimulated	Stimulated	Non-stimulated	Stimulated	Non-stimulated	Stimulated
ATP	6.80 $\pm$ 0.28	4.54 $\pm$ 0.49**	6.72 $\pm$ 0.28	5.09 $\pm$ 0.35**	6.98 $\pm$ 0.35	4.79 $\pm$ 0.25**
ADP	1.12 $\pm$ 0.07	1.21 $\pm$ 0.09	1.05 $\pm$ 0.09	1.43 $\pm$ 0.11	1.10 $\pm$ 0.07	1.15 $\pm$ 0.08
AMP	0.03 $\pm$ 0.006	0.06 $\pm$ 0.009*	0.02 $\pm$ 0.004	0.07 $\pm$ 0.018*	0.04 $\pm$ 0.005	0.09 $\pm$ 0.015**
IMP	0.14 $\pm$ 0.02	1.08 $\pm$ 0.19**	0.13 $\pm$ 0.02	0.42 $\pm$ 0.15*†	0.09 $\pm$ 0.02	0.31 $\pm$ 0.03**††
Lactate	1.25 $\pm$ 0.22	2.06 $\pm$ 0.24*	1.28 $\pm$ 0.27	1.59 $\pm$ 0.41	1.69 $\pm$ 0.05	3.51 $\pm$ 0.38**††††
Glycogen ^a	34.0 $\pm$ 2.8	21.7 $\pm$ 1.9**	32.5 $\pm$ 2.1	26.1 $\pm$ 3.6	33.5 $\pm$ 2.9	13.7 $\pm$ 1.1**††††
Cyclic AMP ^b	559 $\pm$ 49	564 $\pm$ 63	374 $\pm$ 64†	356 $\pm$ 65†	300 $\pm$ 38††	225 $\pm$ 40††

of conditions such as hypertension (Tarazi & Dunstan, 1972; Van Baak *et al.*, 1982). Patients are often encouraged to undertake clinically supervised exercise programmes (Ekblom *et al.*, 1972; Lundborg *et al.*, 1981; Frisk-Holmberg *et al.*, 1985) and there is considerable interest in the possible beneficial and detrimental effects of β -blockade on exercise performance. Muscle fatigue is a common complaint of patients (and healthy volunteers) treated with β -blockers (Epstein *et al.*, 1965; Ekblom & Goldbarg, 1971; Anderson *et al.*, 1979; Pearson *et al.*, 1979; Tesch *et al.*, 1984); however, whether there is a somatic basis for this is a contentious issue.

In the present study we have used ³¹P-n.m.r. and biochemical and physiological analyses to investigate the effects of acute β -blockade, using the non-selective β -adrenoceptor antagonist propranolol, on contractile performance and muscle bioenergetics. In one set of experiments, isometric contraction of the gastrocnemius muscle *in vivo* at 4 Hz was studied. It was demonstrated that intra-arterial infusion of DL-propranolol at 5 min or 35 min before initiation of muscle contraction caused a significant decrease in muscle cyclic AMP concentration in both the stimulated and contralateral gastrocnemius muscles compared with control animals, but had no effect on the changes in muscle performance, intramuscular pH or phosphocreatine concentration observed throughout the protocol using ³¹P-n.m.r. or on any of the metabolites measured in freeze-clamping experiments (Table 2). Therefore, under these conditions, where muscle bloodflow is increased maximally and muscle oxygen consumption is about 70% of $V_{O_{2\max}}$ (Mackie & Terjung, 1983; Connitt *et al.*, 1986), acute β -blockade had no effect on muscle performance or bioenergetics.

There is some evidence from human studies that effects of β -blockade on muscle performance are seen when progressive incremental or discontinuous (i.e. alternate periods of exercise and rest) exercise protocols are used (Lundborg *et al.*, 1981; Twentyman *et al.*, 1981; Tesch & Kaiser, 1983; Tesch *et al.*, 1984). To investigate this possibility, isometric contraction of the gastrocnemius muscle *in vivo* at 1, 2 and 4 Hz for 15 min at each frequency was studied. The stimulation intensities

employed require muscle oxygen consumptions of about 15%, 30% and 70% of $V_{O_{2\max}}$ (Connitt *et al.*, 1986).

Using the incremental protocol, acute β -blockade caused a significant decrease in cyclic AMP concentrations in both the stimulated and contralateral gastrocnemius muscles compared with control animals and marked changes in contractile performance and muscle bioenergetics were seen during stimulation at 2 and 4 Hz (Fig. 4). The key findings were that during 4 Hz stimulation (after prior stimulation at 1 and 2 Hz), β -blockade resulted in a significant 30% decrease in contractile performance, whilst muscle phosphocreatine concentration was 60% higher, and IMP concentration 60% lower, when compared with gastrocnemius muscle of control animals during stimulation at this frequency. In addition, it has been shown that administration of an equimolar dose of D-propranolol did not cause any of these effects, strongly supporting the notion that these effects of DL-propranolol are solely related to its properties as a β -adrenoceptor antagonist.

Fatigue has been defined as 'the inability of a physiological process to continue functioning at a particular level and/or the inability of the total organism to maintain a pre-determined work intensity' (Edwards, 1978, 1983). With respect to the present study where recruitment of 100% of muscle fibres was caused by stimulation of the sciatic nerve at a supramaximal voltage, it was demonstrated that incrementing the stimulation frequency in the presence of β -blockade resulted in greater fatigue at higher work intensities, compared with controls, as the gastrocnemius muscle failed to generate the expected twitch-tension. What is the biochemical basis of the observed 'relative fatigue'? A number of reasons for muscle fatigue have been proposed (see for reviews Edwards, 1978, 1983; Belcastro *et al.*, 1985); these can be summarized as arising from neural, metabolic or contractile malfunction.

Neural malfunction. Fatigue is evident during acute β -blockade when the work intensity is incrementated; however, in similar experiments where muscle stimulation

Fig. 5. Changes in twitch-tension and phosphocreatine concentration in gastrocnemius muscle during stimulation at 1, 2 and 4 Hz in control (▨), propranolol-treated (▤) and pre-stimulated propranolol-treated (□) animals

Values are means $\pm$ S.E.M. for at least seven animals in each experimental group. Time points shown are 2, 8 and 15 min at each stimulation frequency. Statistical significance (Student's *t*-test for unpaired observations) is shown as: * $P < 0.05$, ** $P < 0.01$ for control versus propranolol-treated; † $P < 0.05$, †† $P < 0.01$ for propranolol-treated versus pre-stimulation + propranolol; ‡ $P < 0.05$ for control versus pre-stimulation + propranolol.

was initiated at 4 Hz, 35 min after administration of β -blocker, or the incremental protocol was initiated after a brief pre-stimulation at 4 Hz, fatigue was not observed. In addition, in all experiments, the pulse voltage applied to the sciatic nerve was supramaximal (that is, increasing either the applied voltage or the pulse duration had no effect on the measured twitch-tension) and the measured muscle action potential was normal. All of these observations suggest that the cause of fatigue is not due to neural malfunction.

Impairment of energy supply. Fatigue may be caused by

inability of muscle to maintain energy production to meet an increase in energy demand. β -Blockade counters increases in heart rate and blood pressure upon muscle stimulation (Table 1); furthermore, β -adrenoceptors in the vascular bed of skeletal muscle may be important in peripheral vasodilation during exercise (Hudlická, 1985) and hence, maintenance of muscle perfusion and oxygen and substrate delivery (Christensen & Galbo, 1983). In addition, β -adrenoceptors have been characterized in the sarcolemma of skeletal muscle (Bowman & Nott, 1969; Williams *et al.*, 1984) which are likely to mediate catecholamine potentiation of the effects of Ca^{2+} on

muscle glycogenolysis observed under some experimental conditions (Cohen, 1982; Richter *et al.*, 1982a) Impairment in muscle energy production would not appear to be the primary lesion responsible for the results presented in this study: available substrates were present at concentrations similar to those observed in control animals, plasma glucose concentrations increased by about 10% during muscle stimulation and muscle glycogen was not significantly depleted. Moreover, an impairment in muscle perfusion was not indicated, since intramuscular pH showed identical changes to those seen in control animals and neither intramuscular nor systemic lactate concentrations were increased; furthermore, the steady-state phosphocreatine concentration was higher in contracting gastrocnemius muscles of animals receiving β -blockade than in muscles of control animals.

Contractile impairment. If it is assumed that the concentration of DL-propranolol used in the present investigation was sufficient to block completely β -adrenoceptor agonist stimulatory mechanisms in skeletal muscle, then it must be concluded that catecholamines do not affect contractile performance during stimulation at 4 Hz. However, it would appear that catecholamines do affect contractile performance when exercise intensity is incremented.

Rat gastrocnemius muscle contains approx. 90% type II fibres (Armstrong & Laughlin, 1983) and it has been reported that β -adrenoceptor agonists have a potentiating effect on twitch-tension in fibres of this type (Bowman & Nott, 1969; Nesher *et al.*, 1980); however, neither the mechanism, nor the physiological importance, of this phenomenon has been ascertained. In cardiac muscle, catecholamines exert positive inotropic and chronotropic actions (Katz, 1979; Winegrad, 1984) and these effects have been linked to the phosphorylation of troponin-I and phospholamban which are target proteins for cyclic AMP-dependent protein kinase (Kranias & Solaro, 1982; Kranias *et al.*, 1985; Molla *et al.*, 1985); however, there is suggestive evidence that these mechanisms are not observed in skeletal muscle (Wilkinson & Grand, 1978; Kranias & Solaro, 1982). Another putative regulatory protein is C-protein, which is found in cardiac and skeletal muscle and is phosphorylated by cyclic AMP-dependent protein kinase (Jeacocke & England, 1976; Lim & Walsh, 1986); the precise role of C-protein and the significance of its phosphorylation have yet to be elucidated.

A key finding of the present study was that brief stimulation of gastrocnemius muscle at the highest frequency used (4 Hz) prevented the 'fatigue response' observed in the incremental exercise protocol. Considerable interest has centred on the role of myosin light chain phosphorylation in skeletal muscle since it was reported to be associated with a decrease in the energy cost of contraction (Crow & Kushmerick, 1982). Although some evidence is available suggesting that catecholamines do not affect myosin light chain phosphorylation (Manning & Stull, 1982), we considered it worthwhile to investigate myosin light chain phosphorylation during incremental muscle stimulation in the absence and presence of β -blockade. It was found that stimulation at 1, 2 or 4 Hz did not significantly alter myosin light chain phosphorylation in either the presence or absence of β -blockade (G. D. A. Malloch & D. J. Hayes, unpublished work).

Therefore, in summary, at the present time we believe that fatigue observed during incremental stimulation of gastrocnemius muscle in the presence of DL-propranolol is caused by an as yet undefined dependence of the contractile process upon cyclic AMP-dependent event(s) to maintain maximal contractile force. This may involve regulation of the contractile apparatus *per se* or regulation of the myofibrillar ionic environment. In this context, it is interesting to note that it has recently been shown that potassium homeostasis is under α - and β -adrenoceptor control in skeletal muscle during exercise (Williams *et al.*, 1985).

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